September 5, 2002

SUBSTITUTE SEQUENCE LISTING

<110> Eck, Jurgen
Schmidt, Arno
Zinke, Holger

<120> Recombinant Fusion Proteins Based on Ribosome-Inactivating Proteins of the mistletoe Viscum album

<130> 09282-5

<140> 09/347,064
<141> 1999-07-02

<150> PCT/EP98/00009
<151> 1998-01-02

<150> EP 97 10 0012.0
<151> 1997-01-02

Sub E1

<160> 38

<170> PatentIn Ver. 2.1

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ctcttgcgtc agtctacgat ccccgtctcc gatgcgcaaa gatttgtctt ggtggagctc 180
accaaccagg ggggagactc gatcacggcc gccatcgacg ttaccaatct gtacgtcgtg 240
gcttaccaag caggcgacca atcctacttt ttgcgcgacg caccacgcgg cgcggaaacg 300
catctcttca ccggcaccac ccgatcctct ctcccattca acggaagcta ccctgatctg 360
gagcgatacg ccggacatag ggaccagatc cctctcggta tagaccaact cattcaatcc 420
gtcacggcgc ttcgttttcc gggcggcagc acgcgtaccc aagctcgttc gattttaatc 480
ctcattcaga tgatctccga ggccgccaga ttcaatccca tcttatggag ggctcgccaa 540
tacattaaca gtggggcgtc atttctgcca gacgtgtaca tgctggagct ggagacgagt 600
tggggccaac aatccacgca agtccagcat tcaaccgatg gcgtttttaa taacccaatt 660
cggttggcta taccccccgg taacttcgtg acgttgacca atgttcgcga cgtgatcgcc 720
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<210> 2
<211> 252
<212> PRT
<213> Viscum album

<400> 2
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Met Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu
  1               5                  10                  15

Glu Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly
             20                  25                  30
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Ser Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val
35 40 45

Ser Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly
50 55 60

Asp Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala
65 70 75 80

Tyr Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly
85 90 95

Ala Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe
100 105 110

Asn Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln
115 120 125

Ile Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg
130 135 140

INS E1

Phe Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu
145 150 155 160

Ile Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg
165 170 175

D1

Ala Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr
180 185 190

Met Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln
195 200 205

His Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro
210 215 220

Pro Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser
225 230 235 240

Leu Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro
245 250

<210> 3
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cagttgtggc cctccaagtc caacaatgat ccgaatcagt tgtggacgat caaaagggat 180
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aatgggacca tcatcaatcc aagatccaat ctggttttgg cagcatcatc tggaatcaaa 360
ggcactacgc ttacggtgca aacactggat tacacgttgg gacagggctg gcttgccggt 420

aatgataccg ccccacgcga ggtgaccata tatgggttca gggacctttg catggaatca 480
aatggaggga gtgtgtgggt ggagacgtgc gtgagtagcc aaaagaacca aagatgggct 540
ttgtacgggg atggttctat acgcccccaaa caaaaccaag accaatgcct cacctgtggg 600
agagactccg tttcaacagt aatcaatata gttagctgca gcgctggatc gtctgggcag 660
cgatgggtgt ttaccaatga aggggccatt ttgaatttaa agaatgggtt ggccatggat 720
gtggcgcaag caaatccaaa gctccgccga ataatcatct atcctgccac aggaaaacca 780
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<211> 267
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<213> Viscum album

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1 5 10 15

Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
20 25 30

E1 INS

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
35 40 45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
50 55 60

D1

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65 70 75 80

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
85 90 95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
100 105 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
115 120 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
130 135 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145 150 155 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
165 170 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
195 200 205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
210 215 220

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Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225                 230                 235                 240

Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
                245                 250                 255

Gln Met Trp Leu Pro Val Pro Gly Gly Tyr His
            260                 265
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<210> 5
<211> 72
<212> DNA
<213> Viscum album

<400> 5

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gatgttacat gt                                                      72
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INS E1

<210> 6
<211> 17
<212> PRT
<213> Viscum album

<400> 6

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Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile
  1               5                  10                  15
Ala
```

D1

<210> 7
<211> 756
<212> DNA
<213> Viscum album

<400> 7

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atcacgcttc tccgagatta tgtctcaagc ggaagctttt ccaatgagat accactcttg 120
cgtcagtcta cgatccccgt ctccgatgcg caaagatttg tcttggtgga gctcaccaac 180
caggggggag actcgatcac ggccgccatc gacgttacca atctgtacgt cgtggcttac 240
caagcaggcg accaatccta ctttttgcgc gacgcaccac gcggcgcgga aacgcatctc 300
ttcaccggca ccacccgatc ctctctccca ttcaacggaa gctaccctga tctggagcga 360
tacgccggac atagggacca gatccctctc ggtatagacc aactcattca atccgtcacg 420
gcgcttcgtt ttccgggcgg cagcacgcgt acccaagctc gttcgatttt aatcctcatt 480
cagatgatct ccgaggccgc cagattcaat cccatcttat ggagggctcg ccaatacatt 540
aacagtgggg cgtcatttct gccagacgtg tacatgctgg agctggagac gagttggggc 600
caacaatcca cgcaagtcca gcattcaacc gatggcgttt ttaataaccc aattcggttg 660
gctatacccc ccggtaactt cgtgacgttg accaatgttc gcgacgtgat cgccagcttg 720
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<210> 8
<211> 252
<212> PRT
<213> Viscum album

<400> 8

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Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu Glu
 1               5                  10                  15

Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly Ser
            20                  25                  30

Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val Ser
        35                  40                  45

Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly Asp
    50                  55                  60

Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala Tyr
 65                  70                  75                  80

Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly Ala
                85                  90                  95

Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe Asn
            100                 105                 110

Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln Ile
        115                 120                 125

Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg Phe
    130                 135                 140

Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu Ile
145                 150                 155                 160

Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg Ala
                165                 170                 175

Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr Met
            180                 185                 190

Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln His
        195                 200                 205

Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro Pro
    210                 215                 220

Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser Leu
225                 230                 235                 240

Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro Ser
                245                 250
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D1

<210> 9
<211> 789
<212> DNA
<213> Viscum album

<400> 9

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tgcgtggacg tccgagatga cgatttccgc gatggaaatc agatacagtt gtggccctcc 120
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aagtccaaca atgatccgaa tcagttgtgg acgatcaaaa gggatggaac cattcgatcc 180
aatggcagct gcttgaccac gtatggctat actgctggcg tctatgtgat gatcttcgac 240
tgtaatactg ctgtgcggga ggccactctt tggcagatat ggggcaatgg gaccatcatc 300
aatccaagat ccaatctggt tttggcagca tcatctggaa tcaaaggcac tacgcttacg 360
gtgcaaacac tggattacac gttgggacag ggctggcttg ccggtaatga taccgcccca 420
cgcgaggtga ccatatatgg gttcagggac ctttgcatgg aatcaaatgg agggagtgtg 480
tgggtggaga cgtgcgtgag tagccaaaag aaccaaagat gggctttgta cggggatggt 540
tctatacgcc ccaaacaaaa ccaagaccaa tgcctcacct gtgggagaga ctccgtttca 600
acagtaatca atatagttag ctgcagcgct ggatcgtctg gcagcgatgg ggtgtttacc 660
aatgaagggg ccattttgaa tttaaagaat gggttggcca tggatgtggc gcaagcaaat 720
ccaaagctcc gccgaataat catctatcct gccacaggaa aaccaaatca aatgtggctt 780
cccgtgcca 789

<210> 10
<211> 263
<212> PRT
<213> Viscum album

<400> 10
Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
1 5 10 15

Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
20 25 30

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
35 40 45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
50 55 60

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65 70 75 80

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
85 90 95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
100 105 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
115 120 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
130 135 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145 150 155 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
165 170 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys

```
        195                 200                 205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
    210                 215                 220

Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225                 230                 235                 240

Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
                245                 250                 255

Gln Met Trp Leu Pro Val Pro
            260
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<210> 11
<211> 48
<212> DNA
<213> Viscum album

<400> 11
tcctctgagg tgcgctattg gccgctggtc atacgacccg tgatagcc 48

INS E1

<210> 12
<211> 16
<212> PRT
<213> Viscum album

D1

<400> 12

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Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile Ala
  1               5                  10                  15
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<210> 13
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic gene encoding amino acids 53-78 of human P2 protein

<400> 13
gtaccgggtg gcggtcgtac cgaatccacc ttcaaaaaca ccgaaatctc cttcaaactg 60
ggtcaggaat tcgaagaaac caccgctgac aact 94

<210> 14
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Amino acids 53-78 of human P2 protein

<400> 14
Arg Thr Glu Ser Thr Phe Lys Asn Thr Glu Ile Ser Phe Lys Leu Gly
1 5 10 15

Gln Glu Phe Glu Glu Thr Thr Ala Asp Asn
20 25

<210> 15
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 20:
Synthetic linker cassette for providing modularity
at the 3' end of rMLB delta 1alpha 1beta

<400> 15
caccggtaaa ccgaaccaga tgtggctgcc ggtaccgtag taacgctcct cgtcgaccta 60
gtaaggatcc ctcga 75

INS E1

<210> 16
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 20: amino
acid sequence encoded by portion of SEQ ID NO: 15

D1

<400> 16
Thr Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro
1 5 10

<210> 17
<211> 82
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 21:
Synthetic linker cassette for providing modularity
at the 3'end of rMLB Delta 1alpha 1beta 2gamma
with affinity module ("His-Tag").

<400> 17
ccggtaaacc gaaccagatg tggctgccgg taccgggtgg tggatatcat caccaccatc 60
accactagta actcctcgga tc 82

<210> 18
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Amino acid
sequence encoded by a portion of SEQ ID NO: 17

<400> 18
Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro Gly Gly Gly Tyr His
1 5 10 15

His His His His His
20

<210> 19
<211> 26
<212> DNA
<213> Artificial Sequence

INS E1

<220>
<223> Description of Artificial Sequence:Codon exchange
rMLB D23A

D1

<400> 19
catgtgcgtg gccgtccgag atgacg 26

<210> 20
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1alpha2
(W38A). -

<400> 20
cagatacagt tggcgccctc caagtcc 27

<210> 21
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta (Y68S,
Y70S, Y75S, F79S). -

<400> 21
gctgcttgac cacgtctggc tctactgctg gcgtctctgt gatgatctcc gactgtaata 60
c 61

<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta1
(D235A). -

<400> 22
gggttggcca tggctgtggc gcaagc 26

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

INS E1

<220>
<223> Description of Artificial Sequence:Fig. 22
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 2gamma2
(Y249A). -

D1

<400> 23
cgaataatca tcgctcctgc cacagg 26

<210> 24
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - pT7 EcoRV to
SspI. -

<400> 24
cttccttttt caatattatt gaagcattta tcagg 35

<210> 25
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - pT7 SspI to
EcoRV. -

<400> 25

cttccttttt cgatatcatt gaagcattta tcagg 35

<210> 26
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - pT7 Delta NdeI to
StuI. -

<400> 26
ctttaagaag gagatataca ggcctacgag aggctaagac 40

<210> 27
<211> 33
<212> DNA
<213> Artificial Sequence

INS E1

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB silent NheI. -

D1

<400> 27
gttacctgca gtgctagcga acctacggtg cgg 33

<210> 28
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLA Delta AgeI. -

<400> 28
cccaccagac caccggcgaa gaatatttcc gg 32

<210> 29
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes.

<400> 29

gtttgtatgc ggagagcgtc cctcgagctc tgaggtgcgc 40

<210> 30
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB Delta EcoNI to
AgeI. -

<400> 30
ccgaataatc atcgctccgg ccaccggtaa accaaatcaa atg 43

<210> 31
<211> 11
<212> DNA
<213> Artificial Sequence

INS E1

<220>
<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

D1

<400> 31
tacatatgta c 11

<210> 32
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 32
ccatgataag gatcctctag 20

<210> 33
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 33

caggcctac 9

<210> 34
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 34
cactagtaac tcctcggatc ctctagagtc gacc 34

<210> 35
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Modulator
module peptide

INS E1

<400> 35
Lys Asp Glu Leu
1

D1

<210> 36
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Modulator
module peptide

<400> 36
His Asp Glu Leu
1

<210> 37
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Portion of the
ML propeptide

<400> 37
Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile
1 5 10 15

Ala

INS E1

<210> 38
<211> 13
<212> PRT
<213> Artificial Sequence

D1

<220>
<223> Description of Artificial Sequence:A degradation
product of myelin basic protein.

<400> 38
Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro
1 5 10